Hydration of the lens during the development of galactose cataract

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Changes in hydration of the lens which occur in three arbitrarily defined stages during the development of galactose cataract have been studied. At the onset of the cataractous process the increase in hydration is due to the accumulation of dulcitol. The water content which is only slightly increased in the intermediate stage is accounted for mainly by the retention dulcitol, and to a smaller degree by an increase in salt. In contrast to the early stages, when the cataract matures the large influx of water is no longer attributed to the accumulation of dulcitol but to an increase in NaCl.

The development of galactose cataracts in young rats can be divided arbitrarily into three stages. After rats are placed on a galactose-enriched diet, the first sign of opacities in the lens marks the onset of the initial vacuolar stage of cataract. The late vacuolar stage is the intermediate period between the appearance of vacuoles and of a dense nuclear opacity. The easily recognizable dense nuclear opacity represents the mature cataract stage. During the various stages in the development of a galactose cataract, the most striking and easily discernible change in the property of the lens is an increase in hydration. In the initial stage there is a rapid increase in lens water, which appears to be related to the accumulation of the sugar alcohol, dulcitol. No further major change in hydration occurs as the cataract progresses to the late vacuolar stage. However, when the cataract matures there is a striking increase in water. This report deals with some factors that influence osmotic changes during the development of cataracts in galactosemic rats.

Methods

A description of the glucose and galactose diets and the chemical methods employed have been described in previous reports.1 2 Measurements of the density of the lens were made by the procedure described by Sippel.3 Determination of chloride required two rat lenses which were first dried at 105° C. in the presence of 0.1 ml. of 0.1 N-NaOH and then combusted at 475° C. The ash was dissolved in 1 ml. of 0.1 N HNO3 in 10 per cent acetic acid, and chloride determined in a titrator obtained from the American Instrument Co. Recoveries of known amounts of sodium chloride treated in the same manner as the lens were more than 95 per cent.

In the uptake studies of 42K and 22Na, rat lenses were incubated individually in 4 ml. of a balanced salt-bicarbonate medium.4 The tonicity of the medium was adjusted to 290 mOsm. with NaCl. Lenses were removed after incubation, blotted on filter paper, and weighed. They were
then homogenized in 1 ml. of 10 per cent trichloroacetic acid. An aliquot of the lens filtrate and the medium after incubation were taken and counted in a liquid scintillation counter by the usual method. There was no significant change in the counts per milliliter in the medium of $^{24}$Na or $^{42}$K during the incubation.

Results

The time required for the appearance of the first visible lens changes after onset of the high galactose diet depends a great deal on the age of the rats. The younger the rats the more rapidly opacities develop in the form of vacuoles. With the 50 gram Sprague-Dawley male rats used in these experiments, vacuoles are ophthalmoscopically visible in the equatorial region of the lens after 2 days on the diet. The next clearly definable stage of cataract is the appearance of a dense nuclear opacity. The mature cataract develops usually after rats have been on the galactose diet for 2 to 3 weeks. As shown in Fig. 1, the dense nuclear opacity usually appears suddenly in an overnight period. The changes reported for the late vacuolar stage are actually those observed shortly before the maturation of the cataract after 14 days on the galactose diet.

The changes in hydration of the lens during the three arbitrarily defined stages in galactose cataract are given in Fig. 2 and Table I. When opacities are first visible, the initial vacuolar stage, there is an increase of 30 per cent in the water content of the lens. In the late vacuolar stage the hydration of the cataract is about that observed in the initial vacuolar stage. However, since these control rats are now 2 weeks older than those for the initial vacuolar stage their lenses have a lower water content; consequently, the increase in hydration of the cataract in the late vacuolar stage is 50 per cent above that of the control lens. However, the major change in hydration occurs when the cataract matures. At this stage of cataract the hydration was 130 per cent above normal.

Since dulcitol has been implicated in producing osmotic changes in galactose cataracts, the level of the sugar alcohol was followed in the various stages of the cataract. As the results in Table I show, the dulcitol level remains elevated until the cataract matures and then drops to insignificant levels.

Previously, Gifford and Bellovs observed the reversible nature of the galactose cataract. If the galactose diet is replaced by a normal diet the vacuoles in
the lens slowly resorb until they all disappear (Fig. 3). During the course of formation and resorption of vacuoles, changes in dulcitol content and the density of the lens were followed. As shown in Fig. 4, when vacuoles are first visible there is a drop in the density of the lens, indicating an increase in water. The decrease in density of the lens is accompanied by the accumulation of dulcitol. After the rats are taken off the galactose diet and placed on a normal diet, the level of dulcitol in the lens decreases and the density of the lens simultaneously increases. Thus, the changes in water content parallel the level of dulcitol during the resorption of the cataract.

Since electrolytes are an important factor in determining the degree of hydration in tissues in general, the changes in the levels of cations and chloride during the various stages of cataract were followed. In the initial vacuolar stage of cataract, as shown in Table II, the level of total cations (Na + K) as expressed on lens water basis increases as the cataract progresses. The levels of Na and K also decrease as the cataract progresses, indicating a shift in the electrolyte balance towards a more hydrated state.

Table I. Changes in dulcitol and water content in the lenses during development of galactose cataract. Values are given as the mean ± the standard deviation of the mean

<table>
<thead>
<tr>
<th>Condition of rats</th>
<th>Days on diet</th>
<th>Stage of cataract</th>
<th>Based on 10 mg. dry weight of the lens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose fed</td>
<td>2</td>
<td>Initial vacuolar</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Galactose fed</td>
<td>2</td>
<td>Late vacuolar</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>Glucose fed</td>
<td>14-15</td>
<td>Mature</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

Table II. Electrolyte changes during the various stages of galactose cataract

<table>
<thead>
<tr>
<th>Stage of cataract</th>
<th>Condition of rats</th>
<th>mEq./Kg. lens water</th>
<th>mEq./Kg. dry weight of lens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Na</td>
<td>K</td>
</tr>
<tr>
<td>Initial vacuolar</td>
<td>Glucose fed</td>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Galactose fed</td>
<td>32</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Glucose fed</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Late vacuolar</td>
<td>Galactose fed</td>
<td>30</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Glucose fed</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Mature</td>
<td>Galactose fed</td>
<td>44</td>
<td>125</td>
</tr>
</tbody>
</table>

Rats weighing 50 grams were placed on the glucose or galactose diet. Values are given as the mean ± the standard deviation of the mean.
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Fig. 3. Reversal of galactose cataract. A, Vacuoles are visible in the lens of a rat on the galactose diet for 12 days. B, The number of vacuoles has decreased in the lens of the same rat which after 12 days on the galactose diet has now been fed a normal diet for 6 days. C, The lens is almost clear of vacuoles after this rat has been on a normal diet for 28 days.

Fig. 4. Changes in density and dulcitol content of the lenses during the formation and resorption of galactose cataract.

was lower than that of the controls. However, that there was no net change in the level of total cations is shown by the concentration on the dry weight basis. In the late vacuolar stage the content of Na + K expressed on a unit water basis was lower than that in the control but was elevated above that in the initial stage cataract. A slight increase of 42 mEq. per kilogram dry weight of lens in the level of Na + K was found in the late vacuolar stage cataract over that of the control. In the mature cataract, major changes in cation distribution were observed. The level of Na was high and that of K was low, the concentrations approaching those of the aqueous humor. There was a net increase in Na so that the total cation concentration was approximately 300 mEq. per kilogram dry weight of lens higher than that of the normal lens.

The changes in chloride concentration approximated those observed in the cation levels (Table III). Normally, chloride concentration in the lens is lower than in aqueous humor. The chloride content in the initial vacuolar stage cataract was lower than that of the control lens, but this decrease was apparently due to the increase in water content, for on a dry weight basis the chloride concentration was essentially the same as that found in the control. In the late vacuolar stage there was a net increase in chloride of 25 mEq. per kilogram dry weight, as compared to the increase of 42 mEq. per kilogram dry weight in total cations. In the mature cataract a marked increase in chloride was observed that was close to the increase in total cations of 300 mEq. per kilogram dry weight of lens.

The changes in the activity of the cation pump were followed by measuring the rates of $^{42}$K and $^{24}$Na uptake (Figs. 5 and
The cataract in the initial stage showed only a slight change in the rate of $^{42}$K uptake. A strikingly depressed rate of $^{42}$K uptake was found in the intermediate stage of cataract. There seemed to be no further decrease in the rate of $^{42}$K uptake in the mature cataract. Apparently the transport mechanism of K in the mature cataract is sufficiently active to concentrate K to a level twice that of the medium.

In the control lens a distribution ratio of $^{24}$Na between the lens and medium of 0.15 was observed after 75 minutes of incubation and was not changed after 150 minutes. In the initial stage of cataract the lens appears as effective as the normal lens in excluding Na. However, in the late vacuolar stage the pump mechanism is apparently no longer able to keep up with the rate of entry of Na. In the mature cataract the Na ratio approaches unity, indicating the effectiveness of the cation pump to extrude Na is essentially abolished.

The other remarkable change in the mature cataract was a 40 to 50 per cent lowering in the dry weight. This apparent loss of protein when the cataract matures was previously observed by Patterson and Bunting.

Discussion

In the early phase of galactose cataract there seems to be strong support for the view that the osmotic change is attributable to an increase in sugar alcohol. In previous reports the accumulation of dulcitol was shown to parallel the increase in water in the lens of a galactose-fed rat. In vitro incubation of rabbit lens confirms the relationship between dulcitol retention and water increase. The study of the reversal of the galactose cataract also indicates that changes in hydration are determined by the level of dulcitol. In the initial vacuolar stage of cataract the electrolyte changes are consistent with the view that the movement of water was not accompanied by salt. As a result, there was a dilution of the normal electrolytes without a change.

![Fig. 5. $^{42}$K uptake by the lens in various stages of galactose cataract. Each point is the average of at least eight lenses.](image1)

![Fig. 6. $^{24}$Na uptake by the lens in various stages of galactose cataract. Each point is the average of at least four lenses.](image2)
Table III. Changes in chloride content in the lens during various stages of galactose cataract

<table>
<thead>
<tr>
<th>Stage of cataract</th>
<th>Condition of rats</th>
<th>No. of rats</th>
<th>Chloride concentration (mEq./Kg. lens water)</th>
<th>Chloride concentration (mEq./Kg. dry weight)</th>
<th>Change (mEq./Kg. dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial vacuolar</td>
<td>Control</td>
<td>19</td>
<td>20.0 ± 1.5</td>
<td>33.4 ± 2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Galactosemic</td>
<td>21</td>
<td>14.5 ± 2.4</td>
<td>31.4 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>Late vacuolar</td>
<td>Control</td>
<td>24</td>
<td>17.5 ± 2.8</td>
<td>27.0 ± 5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Galactosemic</td>
<td>10</td>
<td>22.2 ± 4.2</td>
<td>52.0 ± 10.9</td>
<td>25.9</td>
</tr>
<tr>
<td>Mature</td>
<td>Galactosemic</td>
<td>12</td>
<td>91.8 ± 8.5</td>
<td>323.3 ± 30.7</td>
<td>296</td>
</tr>
</tbody>
</table>

Values are given as the mean ± the standard deviation of the mean. The control rats for the late vacuolar and mature stage cataracts were 2 weeks older than the controls for the initial vacuolar stage cataract.

in the absolute amount. Since the contents of electrolytes are not increased and the level of amino acids is lowered, the observed increase in hydration during the initial vacuolar stage of cataract, in all likelihood, is caused by the accumulation of dulcitol.

After the vacuoles are visible and until the appearance of the dense nuclear opacity, there seems to be no major change in lens hydration. Dulcitol content is as high in the cataract of the late vacuolar stage as in that of the initial stage. The retention of dulcitol is probably the main factor that determines the extent of the osmotic change but a slight net increase in salt also indicates the situation is more complicated in the late vacuolar stage of cataract. At this stage further departure from normality is shown by the marked changes in the distribution of cations that are indicated by the loss of K and gain of Na (Table II). These changes are apparently due to a marked decline in the activity of the transport mechanism of cations (Fig. 5 and 6). It appears that the efficiency of the cation pump decreases as the cataractous process progresses, so that Na is no longer effectively excluded from the lens. This results in the continuing exchange of Na for K so that the store of K in the lens is steadily depleted. The results obtained from previous studies on cation transport in the lens1, 19 indicate that when the cation pump is adversely affected, a one to one exchange of Na for K does not necessarily lead to an increase in water. As long as each Na ion that enters the lens is compensated by a loss of a K ion, there is no major change in water content. In the late vacuolar stage a redistribution of cations is taking place because of the depressed activity of the cation pump, and quite possibly because of an increase in permeability, but there is no further major change in water content since the net increase of electrolytes is slight.

The rapidity of the events that take place in the maturation of the cataract is difficult to explain. The transition from the late vacuolar stage to the mature stage occurs suddenly in an overnight period, and the accompanying chemical changes are extremely drastic. The tremendous swelling observed is characterized by an increase in both water and salt. It can be calculated that net increases in Na and Cl can account for the large increase in hydration in the mature cataract.

One explanation may have been a sudden failure of the cation pump, but the results indicate the K transport mechanism is as active in the mature cataract as in the cataract of the intermediate stage (Fig. 5). Since no further decrease in activity of cation pump is observed in the mature cataract, the major electrolyte changes may be explained by a sudden alteration in the permeability barrier of the lens. An increase in membrane permeability could result in a more rapid influx of Na than can be
counteracted by the pump mechanism and would be consistent with the findings of the $^{22}$Na uptake studies. Moreover, if the increase in the influx of Na is no longer compensated by the efflux of K, the entry of Na must then be accompanied by Cl. Since an increase in NaCl creates a hypertonicity, there is a movement of water as well. The inability to retain dulcitol could also be explained by an increase in permeability. The resulting osmotic change is typical of a Donnan or colloidal osmotic swelling. The mechanism responsible for the sudden change in permeability and the appearance of the dense nuclear opacity remains obscure.

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REFERENCES

Discussion
Dr. Bernard Schwartz, Brooklyn, N. Y. The observations by van Heyningen of the accumulation of polyhydric alcohols in various types of sugar cataract have initiated a series of studies attempting to correlate the mechanism of cataract production with the formation and concentration of these alcohols. This paper is one of a series of studies in which the authors have postulated that the initiating mechanism for the production of lens opacities in rats with sugar cataracts is due to the osmotic effect of the sugar alcohols. As they have shown in this study, in the first few days during which rats are on a galactose diet, there is a corresponding increase in dulcitol and water content as well as a decrease in the density of the lens. These processes appear to show a similar rate. In order to substantiate an osmotic mechanism, one has to assume both impermeability of the polyhydric alcohols, which appears to be valid, as well as their existence in an unbound, free state within the cell or lens fiber. One also, of course, assumes that there has been relatively few permeability changes of the cell wall.

The other possibility, to account for the production of opacities, would be a change in the metabolic functions of the lens. This has been considered previously, primarily by Lerman. One crucial index of change in metabolism would be the adenosine triphosphate (ATP) level at the time when little or no opacities were visible as operationally defined by the slit lamp or the ophthalmoscope. The data presented today show that the ATP level only decreases approximately 10 per cent when opacities are first visible at 5 days in the 100 gram rat on a galactose diet. The data of Lerman, with the same technique of ATP measurement but with the 60 gram rat, indicated that at the time when vacuoles are seen first in about 2 days, there was also about a 10 per cent drop in ATP level. The major point here is the criteria used to observe lens opacities, since Lerman stated that lens opacities were first present at 9.5 days. The problem then arises as to whether a 10
per cent drop in ATP level may be critical enough to cause changes in metabolism which would hasten the onset of the cataract, especially by alterations in the transport of cations. Changes in transport would bring about changes in water content of the lens, which could explain the increased hydration rather than an osmotic mechanism. As the authors have indicated, the changes in water content of these lenses were associated initially with no significant change in the sum of the sodium or potassium concentrations. Therefore, this appears to be purely an increase in water content. However, on calculating the change in water content, there is approximately 115 \( \mu \text{M} \) of water brought into the lens per micromole of dulcitol formed (Table IV). This ratio appears rather high.

It is apparent that if an osmotic mechanism is playing a major role here, then a critical concentration of polyhydric alcohol is needed, since van Heyningen\(^5\) has shown that xylitol can accumulate in xylene cataracts up to concentrations of one-fifth of dulcitol levels present in galactose cataracts without the appearance of opacities. Similar results were noted for arabitol at about one-tenth of the level for dulcitol.

One critical factor for the rate of formation of dulcitol is the supply of reduced triphosphopyridine nucleotide (TPNH) for the enzyme aldose reductase which converts galactose to dulcitol. The authors have shown in a previous paper\(^1\) that with the formation of dulcitol there is an increased utilization of glucose through the hexose monophosphate shunt to supply TPNH. The increase in rate for a rat lens in vitro appears to be approximately four times, so that there is a corresponding increase of TPNH formation of eight times. The large utilization of TPNH for dulcitol formation results in changes in the ratio of TPNH to TPN and possibly the ratio of reduced diphosphopyridine nucleotide (DPNH) to DPN. These changes undoubtedly cause shifts in metabolic functions. It thus appears that associated with the formation of dulcitol, there is diversion of the metabolic pathways of the lens. This does not appear to have expressed itself in changes in lactate formation as indicated by the authors' previous data. However, as indicated by Sippel,\(^3\) there are early metabolic changes in glycolysis. Furthermore, studies of oxidative metabolism, especially oxygen utilization, would certainly be of value.

The authors have certainly stated a strong case for the osmotic mechanism in the early development of galactose cataract but it is rare in biologic events for only one cause to be solely acting. Undoubtedly, there are multiple mechanisms concurrently in effect and the causality of anyone of them can only be determined by its sequence in time to a very clear operational definition of lens opacity.

REFERENCES